with J. T. Cash's Compliments

No. 4.

Report on Chlorine, Bromine, Iodine, and Sulphurous Acid as Disinfectants; by Dr. Cash, F.R.S.

From observations of MM. de la Croix and Koch we have already learnt much of the destructive action of halogen elements or their solutions upon the bacillus and spore of anthrax.

The information yielded seemed of such great importance as to make a still more extensive examination of these substances desirable.

I have therefore carried out an extensive series of experiments with the object of determining the lethal limits of the bodies mentioned, not only with regard to anthrax but to human and bovine tuberculosis.

I was further requested by the Local Government Board to extend my observations to sulphurous acid which is already recognised as one of our most efficacious and practical means of disinfection.

Methods adopted.

With the object of rendering the investigation as precise as possible, I have when testing the effect of solutions of these halogens, employed graduated strengths which could be readily controlled by means of occasional standardising. Water solutions of chlorine lose strength with more or less rapidity, solutions of sulphurous acid rapidly, a partial conversion into sulphuric acid as a result of oxidation taking place. Bromine in solution is moderately stable and iodine practically perfectly so.

Using a solution of iodine of known strength as the starting point for the standardising of the other solutions, and taking of it an $\frac{n}{10}$ solution (*i.e.*, 12·653 grms. of iodine per 1000 C.C. distilled water, or 1·27 per cent.), it is easy to satisfy ourselves that an equal volume of standard hyposulphite of soda solution $\frac{n}{10}$ discharges the colour of the iodine, starch being used as the indicator. The hyposulphite being found up to strength, the standardising of chlorine and bromine solutions are effected by estimating their power of liberating iodine from iodide of potassium, starch being as before used as indicator.

Koch places the strength of the solutions of halogens which arrest the development of anthrax bacillus as follows:—

Iodine	1 to 5,000
Bromine	1 to 1,500
Chlorine	1 to 1,500

He further found that spores of anthrax were destroyed in:—

Iodine (1 to 7,000)	1 day
Bromine (2%)	1 to 5 days
Chlorine (freshly made)	1 to 5 days

The original solutions which I employed were:—Of iodine, $\frac{n}{10}$; of bromine, $\frac{n}{20}$; of chlorine, $\frac{n}{20}$.

From these, more dilute solutions were made by careful measurement and controlled by standardisation.

($\frac{n}{100}$ solutions have the following values:—

Iodine	1·27 grm.	in	1000 C.C.	water.
Chlorine	·3537 „	„	„	„
Bromine	·7975 „	„	„	„)

Methods adopted.

The real question at issue is not merely the action of a certain solution having a given per-centage strength, but the action of such a fraction, say of a cubic centimetre containing a known quantity of the disinfectant, upon a given quantity of blood or cultivating medium within which the particular micro-organism is present. Supposing, for instance, that we exposed $\frac{1}{20}$th of a drop of anthrax blood to the action of iodine, we obtain no definite notion of its disinfecting power by stating that the latter was present in the proportion of 1% in the solution unless we know further the actual quantity of the solution available for action upon the micro-organism. (By exposure in a hermetically sealed bulbous pipette the strength of the solution is well ensured during its action, its complete mixture having been accomplished beforehand in a watch glass.) Supposing, to complete the example, that ·1 C.C. of $\frac{n}{100}$ iodine solution is mixed with $\frac{1}{20}$th drop of anthrax blood we then have as iodine actually present, in 1 C.C. ·00127, and in $\frac{1}{10}$th C.C. $\frac{0\,0\,1\,2\,7}{10}$ = ·000127 grm. We can state therefore that this quantity of iodine acting for five minutes upon $\frac{1}{20}$th drop of anthrax blood destroys the contained bacilli, in other words thoroughly disinfects it in a given time.

The experiments I have hitherto made with the haloid bodies and sulphurous acid are in so far double, as they test the disinfectant action of these bodies in solution and as vapours passed through a solution containing the micro-organism to be disinfected.

In the end, it is true that the effect is—as the menstruum becomes more and more highly charged with gas which is dissolved in it,—in both cases that of the exposure of a cultivation of whatever the microbe may be to a disinfectant solution; but it is in the first instance a matter of considerable interest and utility to determine how far the passage of gas through an infusion of some pathogene will destroy its life, when, by reason of the speed of its transmission probably not all but a part only of the gas acts directly upon it. It is in the first instance less a question of the action of a saturated fluid than it is of the affinity of the passing gas, so to speak, for the microbe with which it is in conflict. Even at very slow speeds of delivery of air charged with disinfectant vapour, of say 12–20 bubbles per 1′, it is found that from the first a certain amount of gas, be it chlorine, bromine, or SO_2, passes through the infusion of the pathogene and discharges iodine on the distal side from an iodide of potassium solution. On the other hand, if a substance such as KI., upon which the generated gas acts chemically, liberating iodine, be substituted for the pathogenic infusion, so that we have two vessels containing KI. solution in series, we find that a very rapid discharge of the gas is necessary to decolourise the solution, situated distally; not until all the KI. is decomposed in the vessel into which the gas first passes, does the further vessel begin to show decolourisation likewise.

I will now proceed to consider the action of each of these bodies, and shall quote experiments having a typical result as the best means of illustration because it implies a statement of method as well as of result in every instance :—

And first of these agents *IN SOLUTION*—

A. Chlorine.

Chlorine Solution Anthrax.

On Anthrax.—The general plan has been followed of mixing one part of the blood of an animal just dead of the disease (containing bacilli only)—or one part of spores derived from cultivation—as nearly as possible with 49 of the chlorine solution. When other proportions have been used they will be indicated.

The following experiments with bacilli derived from the blood will serve to illustrate the general method of research :—

Blood bacilli.

(1.) Removed (by puncture with a pipette) blood from the heart of a guinea-pig just dead of anthrax.—(The animal had been inoculated two days previously with bacilli from another guinea-pig.)—Mix 1 minim with 49 times its bulk of $\frac{n}{20}$ chlorine solution (about ·00018 grm. chlorine); and closed up in a bulbous glass pipette, after thorough mixture of blood and solution had been rapidly accomplished in a watch glass. In 5′ break ends off pipette and inoculate guinea-pig.

The guinea-pig lived. This experiment on repetition gave a perfectly uniform result.

(2.) A mixture of the same proportion of blood to the same proportion of a $\frac{n}{50}$th Cl. solution exposed for an equal time gave a similar result, the total Cl. in the droplet being in this case ·00007 grm.

(3.) A more dilute solution, $\frac{n}{100}$ Cl., was prepared and standardised. Of this the same proportion was mixed with the blood of an anthrax guinea-pig which had died within 48 hours after the inoculation of spores—·000035 grm. chlorine was thus present in the solution which was allowed to act on the fiftieth part of a drop of anthrax blood for 75′.

The result here again was negative, the animal showing no ill effect after inoculation.

The same result was obtained when the $\frac{n}{100}$ Cl. solution was applied for 5′ only.

Up to this point the results have been uniform—*i.e.*, an application of 1 part of chlorine water containing ·000035 grm. Cl. is sufficient to destroy within 5′ one fiftieth part of its bulk of anthrax blood.

With a solution of half this strength (*i.e.*, $\frac{n}{200}$) a variable result was obtained.

Thus, in one experiment in which fresh anthrax blood (source, guinea-pig inoculated from blood) was exposed in the proportion of 1–49 of a $\frac{n}{200}$ chlorine solution (=·000018 grm. Cl. in 1 minim) for 5′, 20′, and 40′ respectively,—inoculation, at the end of 5′ was fatal to a mouse, whilst the two mice inoculated after the mixture had stood 20′ and 40′ respectively escaped.

A solution of less strength appeared to be inoperative even when applied for a long time, as the following results will show.

A solution of Cl. $\frac{n}{1000}$ was carefully prepared from a decinormal solution. This solution contained therefore ·000035 grm. Cl. per 1 C.C.

Chlorine Solution.

The fresh blood was taken of a guinea-pig which had been inoculated two days previously with anthrax spores. Three tubes were prepared of this, each containing 1 drop of the chlorine solution (= ·0000035 Cl.) to ·02 minims of blood thoroughly mixed. The drop employed was exactly $\frac{1}{10}$th of 1 C.C. The three tubes were closed, and after 5′, 30′, and 60′ interval, they were used respectively for the inoculation of three guinea-pigs. These three animals were found dead the day but one after, the blood of each being crowded with anthrax bacilli.

The dilution here was therefore so great that disinfectant action was lost, even when exposure of the bacilli had been of considerable duration.

In another experiment in which the exposure of the blood to the action of the same amount of chlorine was continued for 24 hours (at room temperature), the mouse inoculated therewith died of well marked anthrax, but a double repetition of the experiment gave the opposite result.

Anthrax spores.

Spores.—Chlorine solution of the strength of $\frac{n}{50}$ mixed in the proportion of 20 parts to one of a strong cultivation of anthrax spores (beef broth), destroys the spores within 24 hours.

My experiments upon the destruction of spores are not yet completed.

Tubercle.

Chlorine on Tubercular Material.—The following experiment was attended by a usual, but as will be seen by a not invariable result.

Bovine Tuberculosis.—Source.—The spleen of a tubercular guinea-pig which had itself been inoculated from another animal suffering from bovine tuberculosis.

A portion of this spleen was reduced to a condition of pulp in a mortar, the mixture strained through muslin and diluted to 1 C.C. One twentieth part of a drop of this infusion was thoroughly mixed with one drop of $\frac{n}{100}$ freshly controlled chlorine solution. Two tubes were charged with the same mixture and exposed at room temperature. Inoculation took place of two guinea-pigs, one after the mixture had stood for one hour, the second after 24 hours.

The first animal (one hour) rapidly developed tubercular glandular swelling, but the second, 24 hours, showed no ill effect from the inoculation.

The amount of chlorine here present was ·000035 grm., and the solution of spleen employed was a very strong one. It is evident, therefore, that where the chlorine is in excess its action will continue to exert itself until the activity of the tubercle microbe is destroyed, the process of destruction demanding a considerable time for its accomplishment when weak solutions of the disinfectant are used.

I have the record of one experiment in which the conditions of procedure were exactly similar to those above detailed, but in this case though the mixture had stood for 28h. 30m., tuberculosis was induced from inoculation.

Using a strong infusion of a tubercular lung (one lung to 2 C.C. distilled water) I found that 4 minims of a chlorine solution, containing ·0008 grm. chlorine, destroyed the virus in one drop of such lung infusion within 90′.

B.—Bromine.

On Anthrax.—A decinormal solution of bromine contains 7·9 grm. of bromine per 1000 C.C. of distilled water. Such a solution was used as the starting point for the creation of higher dilutions of the haloid.

Bromine Solution.

The strength of the solutions was controlled by means of a standard hyposulphide of sodium solution which had itself been verified by a standardised iodine solution.

The mode of application of the solution resembled that of chlorine which has been already discussed. 49 parts by measure of the standardised bromine were mixed in different experiments with one by measure of fresh anthrax blood, of spore culture, or of strong infusion of a tubercular organ, and set aside in closed pipettes until the time for inoculation had arrived.

Anthrax bacilli.

As regards bacilli of anthrax I may at once begin with a consideration of experiments made with a $\frac{n}{100}$ solution, as anything stronger than this was almost instantly fatal to the bacilli.

Three tubes were prepared each containing one fiftieth of a minim of blood (obtained from a guinea-pig dead in 48 hours after inoculation of bacilli) mixed with a minim of bromine solution containing ·00008 grm. bromine. These tubes were closed, and in 5′, 20′, and 30′ guinea-pigs were inoculated. All of three animals lived.

As regards guinea-pigs I may state in one word, that exposure for 5′ or only for 3′ (the shortest time interval I tested) of the fresh anthrax virus to the $\frac{n}{100}$ bromine solution in the proportions mentioned, was almost invariably destructive of the lethal action of the virus. On one single occasion only, after 5′ exposure, a mouse became infected, but this experiment was the sole exception I witnessed.

A solution of one half this strength (total bromine present ·00004 grm.) when mixed in similar proportions proved itself much less active and did not destroy the virus of the fresh anthrax blood within 30′.

Taking the same amount of a freshly prepared solution, one tenth the strength of that previously employed, *i.e.*, $\frac{n}{1000}$, I found that even on very prolonged exposure its action was practically nil as a means of destroying the anthrax bacillus of fresh blood, ·000008 grm. bromine was present in this instance. Thus after finding that exposure for 5′ had no effect I increased the time to one hour, then to six and finally to 24 hours. But with only one exception occurring after 24 hours' exposure, I found the virulence of the anthrax unmitigated, as evidenced by its fatal effect upon guinea-pigs within 48 hours of the time of inoculation.

When speaking of iodine I shall refer to the plan I adopted for estimating exactly the proportion of blood and disinfectant employed, but I must here give the result of comparative experiments conducted on the principle already indicated.

The mixtures were made of blood and bromine $\frac{n}{100}$th solution in the following proportions. Each stood for 5′–15′ in closed pipettes after mixture had been thoroughly effected, and inoculation of a mouse was then made with each.

(1.) One fiftieth of a minim of anthrax blood with ·00008 grm. Br. for 5′.
(2.) One half „ „ ·00004 grm. Br. for 5′.
(3.) One half „ „ ·00004 grm. Br. for 15′.

The mouse No. 1 lived, but both No. 2 and No. 3 died of anthrax.

Bromine Solution.

Experiments on Anthrax Spores.—These experiments are not yet terminated but I shall give a few which have yielded positive results. The most rapid disinfection of spores I have yet obtained with bromine resulted after thoroughly mixing and allowing to stand for one hour, two minims of bromine solution, containing ·00032 grm. bromine, with one fiftieth of a minim of a beef broth cultivation of spores.

Anthrax spores.

$\frac{n}{100}$ bromine solution mixed with a cultivation of spores in beef broth in the proportion of 49 to 1 is fatal to them if the exposure be continued for a considerable time. Thus,

Of two tubes containing spores (cultivation in beef broth 9 months old) mixed with bromine solution in the above proportions, closed and kept, one at room temperature 19° C., the other at 44·5° C. for 48 hours, both were found on inoculation to have lost their activity.

A much shorter exposure, 18 to 28 hours, is often sufficient for complete disinfection with bromine solutions of the above strength, but one or two exceptions I have witnessed to the uniformity of destructive action even after such considerable intervals of time seem to make a still more protracted exposure necessary for reliable disinfection.

The variability in the resisting power of spores is clearly indicated by these and other experiments which I need not quote here. The chief or most potent cause of this variability is to be found (speaking from my own experience merely) more in the medium of growth than in the age of the cultivation. Doubtless, however, the view entertained by Dr. Klein is a well founded one, that both of these are important factors in modifying spore resistance.

Tubercle

Bromine on Tuberculosis.—The lungs of a tubercular guinea-pig, inoculated with human tuberculosis from another guinea-pig three months previously, were taken and reduced to a pulp in a mortar with a little water; the turbid fluid was then filtered through muslin and diluted with distilled water up to 2 C.C. Of this, about one fiftieth of a minim measured in a capillary tube was mixed with one minim of solution containing ·00008 grm. of bromine. The tube was closed, and in two hours' time inoculation of a guinea-pig was effected. The animal gradually became tubercular and was destroyed.

In another experiment I exposed a mixture of equal parts of a tubercular infusion, prepared as above, and $\frac{n}{100}$ bromine solution for 28h. 30′, after which time inoculation was made of a guinea-pig. In nine days' time it showed unmistakeable glandular enlargement and eventually became tubercular.

Finding that solutions of this strength had but feeble action, I proceeded to a lower dilution of the disinfectant.

An infusion was prepared from the lungs of a tubercular guinea-pig (source of infection a tubercular guinea-pig suffering from human tuberculosis), just killed.

In a large bulbous pipette drawn out at either end, bromine $\frac{n}{20}$ 20 parts (total = ·000 4 br.) was mixed with one part of the strong lung infusion. The contents of the closed pipette after standing 60′, at room temperature, were used for inoculation; the inoculated guinea-pig did not show any enlargement of the inguinal glands, and though kept under observation for three months, no sign of tubercular disease made its appearance.

C.—Iodine.

A normal solution of iodine contains 127 grms. of iodine per 1000 C.C. distilled water.

A decinormal solution was used for the preparation of higher solutions, the strength of these being controlled by a standard hyposulphite of soda solution.

Iodine Solution.

Iodine on Anthrax.—All solutions containing more iodine than an $\frac{n}{100}$ solution are almost instantaneously fatal to the anthrax bacillus, when mixed in proportion of 49 to 1 of blood.

Anthrax bacilli.

The general statement may be made that solutions of this strength mixed with a bulk of fresh anthrax blood equal to $\frac{1}{49}$th of their own, render the anthrax inert in 3′ (the shortest time interval examined).

The virus is equally inert towards guinea-pigs and white mice. As example I quote an experiment.

One fiftieth of a minim of anthrax blood of a guinea-pig just dead of the disease was mixed with one minim of iodine solution, containing ·000127 grm. of iodine. In 5′ inoculated a mouse. The mouse lived.

The question of the actual amount of anthrax blood which may be disinfected by a given quantity of iodine has closely occupied my attention, and I have made several experiments with the view of ascertaining the precise amount needed of the latter.

I found the best and most accurate plan to be, to employ long pieces of capillary tubing mounted on paper scales, divided into fiftieths, one hundredths, &c. By drawing into such a tube one part of blood, then 49 of a standard iodine solution, blowing the contents out, mixing thoroughly and allowing to stand in covered watch-glass for a given time, I was able to make my observations with extreme delicacy. The total contents of the capillary tube were estimated by recharging and emptying the tube repeatedly till 1 C.C. had been collected, and from this reckoning the total actually present in the pipette.

Experiment.—Mixed in the proportions of—

(1.) 1 of anthrax blood to 49 of $\frac{n}{100}$ iodine.

(2.) 25 of blood to 25 iodine $\frac{n}{100}$ solution.

(3.) 49 of blood to 1 of iodine $\frac{n}{100}$

In each case the mixture stood exactly 5′ before inoculation was made.

In (1.) ·00013 grm. Iodine was present, and $\frac{1}{30}$ minim blood.

In (2.) ·00007 grm. Iodine, and $\frac{1}{2}$ minim blood.

In (3.) ·00003 grm. Iodine, and 1 minim blood.

The virus was entirely destroyed in 5′ in No. 1, but inoculations of mice with Nos. 2 and 3 were fatal to the animals.

I found it necessary, therefore, to make variations in the amount of blood employed less extensive, and repeated the experiment, using—

(1.) One of blood to 49 $\frac{n}{100}$ iodine, ·000127 grm. iodine to one fiftieth drop of blood.

(2.) Four of blood to 46 $\frac{n}{100}$ iodine, ·00011 grm. iodine to half drop of blood.

(3.) Eight of blood to 42 $\frac{n}{100}$ iodine, ·001 grm. iodine to one drop of blood. After 5′ exposure three mice were inoculated.

The result in this case gave evidence that I had arrived at the point at which iodine ceased to disinfect the indicated bulk of blood.

Nos. 1 and 2 lived. No. 3 died of typical anthrax.

Iodine Solution.

The following experiment, in which guinea-pigs were employed instead of mice, had a very similar issue, complete disinfection of only a small proportion of blood taking place in 6′. In each instance the amount of iodine present was exactly equal.

1. 1 part of anthrax blood with 49 of iodine solution containing ·000127 grm. iodine for 6′.
2. 20 parts of anthrax blood with ·000127 grm. iodine for 6′.
3. 40 parts of anthrax blood with ·000127 grm. iodine for 6′.

As a result of inoculation, Nos. 2 and 3 died of anthrax. No. 1 escaped.

It was found further that whereas $\frac{n}{100}$ iodine destroys the virus in $\frac{1}{40}$ part of its bulk of anthrax blood, exposure of this strength of solution with equal bulk of blood did not destroy the virus within 40′ (limit of observation).

I quote one more experiment with an iodine solution $\frac{1}{10}$ the strength only of that just employed.

The mixture here was with one minim of $\frac{n}{1000}$th iodine solution (·0000127 grm. Iodine); the tube was closed and kept at 18° C., and inoculation of a mouse took place after the lapse of 24 hours. The inoculated mouse died of anthrax within two days' time.

Tubercle.

Iodine in Tuberculosis. (*Human Tuberculosis.*)—A strong solution of iodine $\frac{n}{20}$ was found to be rapidly fatal to the virus of tuberculosis when mixed in the proportion of 20 parts of the former to one of the latter (strong infusion).

Thus in one experiment in which the total iodine present was ·0008 grm., it was found that the infective power of one fiftieth of a minim of the lung infusion was destroyed within an hour, inoculation at the end of that time remaining without result upon the animal (guinea-pig).

Experiment.—One lung of a guinea-pig (inoculated from tubercular guinea-pig) was broken up in a mortar, the fluid strained through muslin and diluted to 1 C.C. Half a minim of this solution was mixed with half a minim $\frac{n}{100}$ (·00006 grm.) iodine solution, and allowed to remain in a closed tube for 24 hours. At the expiration of this time a guinea-pig was inoculated. This animal escaped infection.

The result was not uniform however. I have before me the record of a similar experiment in which the time of exposure of the virus to the action of a similar amount of iodine was 28h. 30′, and in this case tuberculosis supervened in the inoculated guinea-pig.

D.—Sulphurous Acid.

Sulphurous Acid Solution

Solutions of this gas rapidly lose strength. Oxidation of the acid further renders the employment of anything but freshly prepared solutions useless for experimental purposes of a quantitative nature.

As far as possible I obviated this disadvantage by using a fresh preparation of the standard solution. Before every experiment it was

necessary to make a control estimation of the strength of the solution and to raise it to the desired standard. Iodine was used as the standardiser, boiled starch as the indicator.

Anthrax bacillus.

The *anthrax bacillus* perished rapidly—almost instantaneously in such solutions as $\frac{n}{20}$ or $\frac{n}{30}$ of the acid—employed in the proportion of 49 to 1 of the blood.

When, however, a solution of $\frac{n}{50}$ was employed, the uniformity of the result was lost, and many cases of failure of the disinfectant were recorded.

Experiment I.—Blood of a guinea-pig just dead of anthrax caused by spore inoculation was mixed in the proportion of 1 in 32 of the $\frac{n}{50}$ solution of sulphurous acid. Three bulbous pipettes each containing ·001 grm. of the acid, into all of which one minim of fresh blood had been introduced, were then closed, and inoculation of animals (guinea-pigs) took place after 1 hour, 2 hours 30 minutes, and 24 hours respectively. All these animals survived, and this result obtained equally whether the disinfectant acted at room temperature or at 37° C.

When the proportion of blood to acid solution was increased, and the length of exposure shortened, a fatal result from subsequent inoculation was not infrequently observed. Thus,

Experiment II.—Fresh blood from guinea-pig, dead after spore inoculation, in the proportion of 1 in 50 of the acid solution, closed in bulbous pipettes till the time of inoculation arrived.

Total acid present = ·0001 grm.

After 25′ (the tube having been kept at 18° C.) inoculation of a guinea-pig (*a*) was performed; at 60′ from an exactly similar tube a mouse (*b*); and at 100′ a second mouse (*c*) was inoculated. After the lapse of two days, all the animals were found dead, the blood in each case abounding with anthrax bacilli.

A longer exposure, from 2–4 hours, under similar conditions was usually found to have been fatal to the anthrax bacilli, as shown by their failure to infect mice and guinea-pigs.

More dilute Solution.—Exposure for 24 hours of the fiftieth part of a minim of fresh blood to the action of 1 minim of solution containing ·0000032 grm. of sulphurous acid.

Death of mice resulted from inoculation with this solution, their blood being crowded with anthrax bacilli.

Exposure of $\frac{1}{50}$th minim of anthrax blood to the action of a drop of solution containing ·000082 grm. sulphurous acid, only results in the destruction of the bacilli of the former when it is long continued.

Thus, after 35′ exposure of such a mixture, death of an inoculated animal (guinea-pig) usually ensues.

In a strictly quantitative experiment, such as I have described under Iodine, it was found that—

- ·00012 grm. sulphurous acid destroyed the virus in $\frac{1}{50}$th minim of anthrax blood in 5′.
- ·0001 grm. sulphurous acid did *not* destroy $\frac{1}{2}$ minim of anthrax blood in 5′.
- ·00001 grm. sulphurous acid did *not* destroy 1 minim of anthrax blood in 5′.

Further that ·00002 grm. sulphurous acid did *not* destroy $\frac{1}{50}$th minim of anthrax blood in 5′.

Sulphurous Acid Solution.

Anthrax Spores.—·0004 grm. of sulphurous acid disinfects one fiftieth of a minim of an anthrax spore cultivation within 24 hours, but after the mixture has stood for one hour only the spores are still active.

I have not as yet been able to pursue this subject further.

Tubercle.

Sulphurous Acid on Tuberculosis.—In view of the great resistance of tubercular virus, I commenced at once the examination of the strong solution of sulphurous acid, varying the time of exposure of the former to the action of the latter.

From the lungs of a freshly-killed guinea-pig (human tuberculosis) a grumous fluid was obtained by pounding in a glass mortar; this fluid was strained off and diluted to 10 C.C. with distilled water. To 2 C.C. of this ·0082 grm. sulphurous acid was added and thoroughly mixed. The mixture was divided into two portions and transferred to two bulbous pipettes which were then closed. After incubation for one hour and 24 hours respectively (at 37° C.) two guinea-pigs were inoculated. Although the former of these animals showed a slight temporary glandular swelling, this soon disappeared, both animals though kept for two months under observation remaining healthy. In each experiment ·0041 grm. $H_2 SO_3$ was present.

From a freshly-killed guinea-pig suffering from human tuberculosis, the lungs were removed, reduced to a pulp in a mortar, the fluid filtered off and diluted to 10 C.C.

Of this, 1 CC. was added to ·0041 grm. sulphurous acid; was mixed thoroughly, introduced into a large closed pipette, and exposed for 24 hours to a temperature of 37° C.

The tubercular material was found to be entirely inactive in producing the disease. The control guinea-pig inoculated with the original infusion became rapidly tubercular.

Passing to more dilute solutions of the disinfectant it was found that equal parts ($\frac{1}{2}$ minim) of $\frac{n}{100}$ acid solution, and a strong infusion (2 grms. to 2 C.C.) of tubercular lung could be mixed together without the activity of the virus being destroyed in 28 hours.

Destruction of Pathogenes by transmission of *GASEOUS* Chlorine, Bromine, and Sulphurous Acid.

GASES.

For a description of the general method of examination of the action of the haloid gases and sulphurous acid, I must refer to my report* of last year on the disinfectant action of ozone and to the diagram it contains, the main difference being that in the present series of experiments the place of the ozone generator is taken by a flask for holding strong bromine, chlorine solution, etc., or introduced for the purpose of generating sulphurous acid (sulphuric acid, and carbon or copper). Air passed from a Pepys gas holder and through this flask, emerged charged to some extent with the gaseous contents of the latter, and if necessary was further washed in a second bottle. The experiment commenced by conducting the issuing gas through a solution of the pathogenic sub-

* Report of Medical Officer to the Local Government Board, 1885.

stance to be acted upon, introduced into a nitrogen bulb. From time to time, usually at the beginning and end of an experiment, this bulb was replaced by another which contained fresh acidulated solution of iodide of potassium, in order that the amount of escaping gas might be estimated for a given time by the liberation of iodine as calculated by subsequent titration with a standard hyposulphite of soda solution. In a few experiments estimation of the total gas passing from beginning to end of the experiment was attempted by placing a second nitrogen bulb containing acidified potassium iodide solution distally as regards the first bulb; the object being by subtracting the iodine liberated in this from the calculated total entering the first bulb to arrive at the total gas absorbed in the latter. This estimation is difficult to carry out with sufficient accuracy to make the results perfectly trustworthy, and as I remarked in my report on the action of ozone, even if the figure given was absolutely correct, it would still be open to question, how much of the gas lost in transit through the first bulb had been spent in acting upon elements, chiefly albuminous, other than micro-organisms which are present; and how much spent on the micro-organisms themselves.

One point I have endeavoured to obtain information upon is this: If a stream of air laden with a certain ascertained amount of disinfectant gas be passed with a given speed through a solution containing pathogenic micro-organisms, how soon will the disinfectant's action make itself felt as evidenced by the destruction of such pathogenes? If we bring two nitrogen bulbs containing iodide of potassium solution into connexion distally with a bottle containing chlorine solution, through which air is slowly forced to them from a Pepys gas-holder, we observe that in the bulb nearer to the gas supply, the salt (iodide of potassium) is rapidly decomposed, iodine being set free, but in the second bulb no decomposition whatever occurs, at any rate for a considerable time, the fluid remaining perfectly colourless.

If now we substitute for the proximal nitrogen bulb another containing, say, an infusion of tubercular lung, and continue the circulation of air charged with chlorine through this and through the distal KI. bulb, we notice almost immediately a liberation of iodine in the latter; this simple experiment shows us that an escape of the gaseous disinfectant takes place before its maximal action has been attained (as demonstrated by experiment), the maximal action for our purpose being death of all micro-organisms, coagulation of other albuminous bodies, etc. Whilst in its passage through the bulb a portion of the chlorine acts on the contents causing certain effects by liberation of oxygen, a portion remains behind in general solution having a potentiality for further action, and still another portion passes away unchanged without having produced any definite disinfectant action. Small quantities of the nascent oxygen are probably lost in the same manner.

There is such a loss to be considered in the action of all gaseous disinfectants in other than strictly closed spaces, and it must be reckoned with in all our attempts at estimation of the probable destructive power of this or that agent. How great the excess of disinfectant must be in order that under given circumstances of application, renewal, intimate mixture, &c., its effect may be thorough and complete, is a very wide and important question which I can merely indicate in this place.

Chlorine as a GASEOUS Disinfectant.

As I have already stated we must consider that the virus present in any infusion traversed by a disinfectant gas which is capable of

absorption by the infusion, is exposed, not only to the action of passing gas which is escaping as such; but also to the continued action of a solution of the gas which during the experiment is constantly increasing in strength till saturation occurs.

Chlorine Gas.

The following notes will serve to illustrate the details of an experiment and its results.

Tubercle.

Took one *tubercular* lung of a guinea-pig killed when suffering from human tuberculosis. The lung was studded with translucent pearls, the spleen enlarged, and like the liver slightly necrotic. The lung was broken up in a mortar with a little distilled water, the fluid strained through muslin, diluted up to 10 C.C. and transferred to a nitrogen bulb.

Air was passed slowly—10 bubbles per minute,—from a Pepys gas holder through a bottle with the proximal tube dipping beneath the surface of a considerable volume of $\frac{n}{20}$th chlorine solution. The second tube communicated by means of an air-tight joint with the nitrogen bulb. The chlorine coming over was determined by liberations of iodine from a slightly acidulated KI. solution—

0′, circulation commenced through the nitrogen bulb containing tubercular material.

10′, infusion has changed much in colour, is now of turbid appearance, deep brownish red. A sample was taken and at once inoculated into a guinea-pig.

The circulation of the gas was interrupted for only a few seconds, and was then continued for 40 minutes more without stoppage.

At the expiration of this time the infusion was of a deep muddy brown colour. A second guinea-pig was then inoculated.

The total chlorine passed through the solution as estimated by the discharge of colour of the liberated iodine by hyposulphite of soda solution was before the first inoculation, ·016 grm.; before the second, ·064 (·064 + ·016 = ·08 grm.).

In spite of this relatively large discharge of chlorine both animals became tubercular, and when killed the usual appearances were found in their organs.

I will now briefly refer to two other experiments which are selected as showing the successful destruction of the *tubercular* virus by chlorine.

The lung employed was that of a guinea-pig suffering from human tuberculosis, and the preparation of its infusion was the same as in the last experiment.

A variation was, however, made in this experiment by generating larger quantities of chlorine from hydrochloric acid and manganese dioxide under gentle heat and conducting the gas through a small wash bottle originally containing water, before leading it through the nitrogen bulb.

A control animal was inoculated with the lung infusion before the circulation of the disinfectant was commenced. An estimation of the escaping gas was made as before, and then the nitrogen bulb with its contained infusion, was put into connexion with the second bottle.

Inoculations were made after the passage of air laden with chlorine at the rate of 16 bubbles per 1′, for (*a*) 2′, for (*b*) 6′, and for (*c*) 10′ respectively. The issuing chlorine was again estimated at the end of the experiment.

It was calculated that before the first inoculation ·0710 grm. of chlorine had been passed.

In all before the second (*b*), ·213 grm.

In all before the third (*c*), not more than ·335 grm.

But as the third estimation of chlorine showed a slight falling off in the evolution of the gas, the total may have been somewhat less.

The control guinea-pig and the guinea-pig (*a*) became distinctly tubercular (though some delay in the development of glandular swelling appeared to occur in the latter).

The other two (*b*) and (*c*) guinea-pigs escaped infection altogether.

Chlorine Gas.

There is only one conclusion to be drawn from this experiment, namely, that air strongly laden with chlorine gas can rapidly disinfect or destroy a great mass of tubercular virus by transmission through a solution of the latter.

Tubercle.

The details of the next experiment are very similar to the last. Chlorine was generated from dioxide of manganese and hydrochloric acid, a flask containing them being exposed to a steady heat on a sand bath. It was first of all ascertained that at the speed at which chlorine was carried over through two bulbs each containing acidulated iodide of potassium solution, the proximal bulb, *i.e.*, that next the chlorine bottle, became rapidly a deep red brown from liberated iodine, whilst the distal bulb remained perfectly colourless. On subsequent titration it was found that ·019 grm. chlorine was coming over in 1′ 30″. This discharge remained constant throughout the short experiment, as was shown by a second estimation of chlorine at its termination.

The nitrogen bulb (containing a strong infusion of one lung of a guinea-pig killed when suffering from human tuberculosis), was now rapidly substituted for the proximal iodine bulb, and the circulation of chlorine continued. At the same time a control animal was inoculated.

In 90″ the infusion was highly turbid and the distal (KI) bulb dark brown from liberated iodine.

In 4′ a guinea-pig (*a*) was inoculated.

In 9′ a second guinea-pig (*b*) was inoculated.

As I have said, the chlorine passing in part unchanged into the distal bulb, which was twice replaced by fresh iodide of potassium bulbs during the experiment, liberated in them free iodine, and the extent of decomposition was subsequently estimated by titration with hyposulphite of sodium.

I found that the—

Total chlorine passing into distal bulb, was in :—	Total generated and passing into lung infusion, was in :—
9′ about ·12 grm.	9′ ·1616 grm.

Result.—The control animal became rapidly and distinctly tubercular and was destroyed.

The animals (*a*) and (*b*) both escaped infection.

The result of the last chlorine experiment I shall quote must be in brief, as this paper is already of a length which can only be justified by the importance of the subject, and the necessity which I have generally recognised for minutely recording processes adopted as well as the results arrived at.

In this instance, in which the infusion of a whole lung of a tubercular guinea-pig (human tuberculosis) was introduced into a nitrogen bulb, the following amounts of chlorine were generated and passed into the infusion.

In 2′ ·0355 grm. Chl., guinea-pig (*a*) inoculated.

In 6′ ·0768 grm. „ „ (*b*) „

Total before (*b*) inoculated = ·1123 grm. Chl.

Result.—The control guinea-pig rapidly became tubercular and was destroyed.

Guinea-pig (*a*) also became tubercular.

„ (*b*) after long observation was found to have escaped.

Bromine as a GASEOUS Disinfectant.

Bromine Gas.

The principle of experiment was here the same as in the case of chlorine, with the exception that air was simply passed over strong solutions of bromine before its admission to the tubercular infusion. In order to limit the amount of bromine coming over, the flask containing it was placed in a vessel containing ice.

Even under these circumstances large quantities of bromine passed into the infusion and disinfection was rapidly accomplished.

I quote two experiments.

Tubercle.

Infusion of one lung of guinea-pig just killed, suffering from human tuberculosis, prepared in the usual manner; air passed slowly (12 bubbles per 1′) over pure bromine in a flask surrounded by ice, and thence passed into the nitrogen bulb containing infusion of lung.

Estimations of decomposition of iodide of potassium were made before and after experiments by titration.

After air laden with bromine had passed through the infusion for 8′, a sample was taken, and a guinea-pig (*a*) inoculated.

After 24′ more, another sample was taken of the dirty reddish-brown deposit which had fallen to the bottom of the bulb, and at once inoculated into guinea-pig (*b*).

Before the first inoculation ·496 grm. of bromine had been passed into the infusion.

The control guinea-pig become infected with tuberculosis, but both (*a*) and (*b*) guinea-pigs escaped.

Anthrax Spores.

Bromine Gas on Anthrax Spores.—A well grown cultivation of anthrax spores in about 3 C.C. beef broth was diluted with distilled water and introduced into a nitrogen bulb. A control mouse was inoculated with the mixture. An estimation of bromine passing from the flask (see last experiment) was made, and the anthrax spore infusion was then put in position and circulation commenced.

After ·284 grm. bromine had come through in 15′, inoculation of a mouse (*a*) was made, and after 1·05 grm. more had passed in the succeeding 45′ (1·337 grm. in all) a second mouse (*b*) was inoculated, in both cases the deposit at the bottom of the bulb being taken for the purpose.

The control mouse died of anthrax in two days. Both *a* and *b* mice escaped—the anthrax spores having been destroyed by the bromine.

Sulphurous Acid *GAS*.

Generated by heating together sulphuric acid and sulphur or carbon, fresh acid being from time to time supplied through a dipping thistle funnel. At all times it was found that even with the slow discharge of gas (12 bubbles per 1′) a certain quantity of sulphurous acid passed through the solution unabsorbed.

Half of one tubercular lung of a guinea-pig suffering from human tuberculosis was broken up in a mortar with distilled water, and diluted to 15 C.C. The infusion was placed in a nitrogen bulb, and air strongly laden with sulphurous acid bubbled through water was passed through the infusion.

Sulphurous Acid Gas.

As estimated by the liberation of iodine the gas was rapidly evolved, in 6′ no less than ·1052 grm. having come over, and in 12′ nearly double that quantity; in the second 6′, however, there was a slight falling off in the quantity, but, as will be presently seen, this later irregularity did not affect the result as the virus had been already destroyed.

Guinea-pigs were inoculated at the end of 6′ (*a*), and 12′ (*b*) respectively.

Tubercle.

The control animal became tubercular.

Both (*a*) and (*b*) escaped infection.

I will quote, in concluding this portion of the subject, only one more experiment, which demonstrates more clearly the time at which destruction of the tubercular virus occurs.

In this instance the same proportion of tubercular lung was used in the infusion as in the preceding experiment.

Sulphurous acid was generated from sulphuric acid and carbon moderately heated. The discharge of sulphurous acid was steady and moderately rapid, occurring at the rate of ·0087 grm. per 1′.

In one and a half minutes after the circulation had commenced a sample of infusion was taken for inoculation (*a*), after 20′ a second sample was taken (*b*), and a third (*c*) after 40′. All these samples were at once used for the inoculation of guinea-pigs. The results were as follows:—

(*a*.)	·013 grm. sulphurous acid.	Animal rapidly became tubercular.
(*b*.)	·18 „ „ „ „	escaped infection.
(*c*.)	·36 „ „ „ „ „	„

Anthrax bacilli.

Action of Sulphurous Acid Gas on Anthrax Bacilli.—Took blood of guinea-pig dead in 48 hours after the inoculation of anthrax spores, and placed five drops diluted to 3 C.C. in test tube through which sulphurous acid was rapidly passed. Sulphurous acid gas was liberated at the rate of ·0077 grm. per 1′. I removed a sample at the end of 15″ and a second one at the expiration of 2′. Each sample was immediately inoculated into a mouse.

(*a*.) Inoculation after ·0019 grm. had passed. Mouse died of anthrax on third day.

(*b*.) Inoculation after ·0154 grm. had passed. Mouse escaped infection.

With Iodine, I have been unable to perform more than two or three experiments of this nature, and must therefore leave the consideration of this substance for the present.

Summary.

The experiments I have quoted are far from exhausting the total number performed. I select them merely as typical, believing that there is advantage in giving them in detail, rather than in making a general statement of results which can scarcely be brought into exact contrast with each other as the circumstances of the experiments must vary within certain limits.

It is necessary to state that the tubercular lungs employed for experiment were taken from animals in which the disease was developed (gray pearls scattered at intervals throughout the lung) but not far advanced.

To enter into the question of the relative values of the disinfectants considered would be to recapitulate a large part of this paper. Employed

in solutions, standardised according to the atomic weight, the disinfecting properties of bromine, chlorine, and iodine, do not largely differ from one another, but they are still capable of arrangement in the inverse order as regards their activity. Sulphurous acid is most valuable in the gaseous form, as its solutions rapidly change their character and thereby their disinfectant property is impaired.

The impotency of very small quantities of any of these disinfectants to destroy such a virus as that of anthrax, when applied even for a very long time, is a matter of no small practical import.

The rapid disinfection by a stronger solution is the most reliable and advisable method of their application, and it is evident that when they are called upon to act in antagonism to some animal virus in the presence of even small quantities of other albuminous material, such virus is apt to escape unscathed, the disinfectant failing in its action by reason of its insufficient proportion to the amount of inert and harmless bodies upon which it is also capable of acting.